Automated Chemiluminescent Assay for C-Peptide

Robert W. Hardy, Michael Cohn, and Robert J. Konrad*

University of Alabama at Birmingham, Birmingham, Alabama

C-peptide is secreted in equimolar concentrations with insulin, and is often measured to assess pancreatic β -cell function. C-peptide analysis is most often performed by radioimmunoassay (RIA) which has several disadvantages. We evaluated an automated, chemiluminescent immunoassay for C-peptide in terms of precision, linearity, interference, and correlation with a RIA method. The chemiluminescent assay demonstrated acceptable correlation with the RIA method (slope = 0.82, y-intercept = 0.88 ng/ml, r-

value = 0.97). Between-run CVs ranged from 8 to 9%, which compared well with the RIA method. Linearity extended beyond the manufacturer's recommendations and recovery ranged from 87 to 112% across the concentrations tested, with a slope of 1.007. No significant interference was noted with hemoglobin, bilirubin, or triglyceride. Overall this method compared favorably with the RIA method and offers an alternative to RIA for the analysis of C-peptide. *J. Clin. Lab. Anal.* 14:17–19, 2000. © 2000 Wiley-Liss, Inc.

Key words: C-peptide; immunoassay; diabetes; insulin; islets

INTRODUCTION

C-peptide is produced in pancreatic β -cells by cleavage of proinsulin into insulin and C-peptide. C-peptide is secreted at an equimolar concentration with insulin (1). It was initially believed that C-peptide lacked any biological activity but recent studies have questioned this idea (2,3).

The quantitative measurement of C-peptide is used largely for the following purposes: (1) differentiation between factitious hypoglycemia and hypoglycemia due to hyperinsulinism; (2) differentiation between type-1 and type-2 diabetes mellitus in certain situations; and (3) assessment of β -cell function in the early stages of type-1 diabetes mellitus. Measurement of C-peptide has advantages over insulin measurement in that hepatic metabolism is negligible and it has a constant peripheral clearance at different plasma concentrations and in the presence of variable plasma glucose concentrations (4–7).

The Diagnostics Products Corporation (DPC, Los Angeles, CA) Immulite® fully automated chemiluminescent immunoassay for C-peptide measurement represents an alternative to radioimmunoassay (RIA) for the quantification of this peptide. Advantages of the chemiluminescent assay over RIA include the use of nonradioactive materials and reduced turnaround time. In this study, we assessed the performance of the DPC Immulite® C-peptide assay in terms of precision, linearity, interference, and correlation with a RIA method.

MATERIALS AND METHODS

The DPC assay consists of a solid-phase enzyme immunoassay that can measure C-peptide concentrations in serum

or heparinized plasma. The assay was developed for an automated, continuous, random-access system (DPC, Immulite®). It consists of enzyme-labeled C-peptide molecules that compete with C-peptide molecules from the sample for purified polyclonal antibodies immobilized on polystyrene beads. After a centrifugal wash, a chemiluminescent substrate is added, and the emission of light is measured by a luminometer. The emission of light is inversely proportional to the concentration of C-peptide in the sample (8).

For the method comparison we performed C-peptide measurements in fasting hospital patients. The same samples were independently analyzed for C-peptide using a double antibody RIA, also from DPC. In addition, we compared four samples that were below the lower level of detection of the RIA method.

For linearity, we used a high-level (10.2 ng/ml) human serum-based calibrator sample from DPC and measured C-peptide concentrations at the following dilutions: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. The limit of detection was determined using the zero calibrator which was analyzed ten times. Precision studies were performed using two levels of lyophilized controls. Within-run precision was performed on 10 consecutive replicates while between-run precision was calculated using data from 20 runs.

*Correspondence to: Robert J. Konrad, M.D., Department of Pathology, University of Alabama at Birmingham, P230G West Pavilion, 619 South 19th Street, Birmingham, AL 35233-7331. E-mail: rkonrad@path.uab.edu

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Interference studies were performed using three serum pools spiked with increasing amounts of either bilirubin (Sigma, St. Louis, MO), hemoglobin from a hemolysate prepared from osmotically-shocked heparinized blood, or triglyceride from Liposyn, a commercial fat emulsion (Abbott, Abbott Park, IL). C-peptide concentrations were compared to the original serum pool plus the vehicle and the percentage change calculated for each concentration. Five to seven concentrations each of bilirubin, hemoglobin, or triglyceride were tested at concentrations ranging from 1.0 to 36 mg/dl, 1 to 9 g/l, and 157 to 2317 mg/dl respectively. The concentration of C-peptide used for the interference studies ranged from 6.3 to 8.4 ng/ml.

RESULTS

The results obtained using the Immulite® C-peptide assay demonstrated acceptable correlation with the RIA reference method. The r -value was 0.97 with a y -intercept of 0.88 ng/ml, and a slope of 0.82 (Fig. 1). The positive intercept indicates a possible bias with the Immulite® method. In order to determine how well the two methods correlated around the lower level of detection (LLD) we compared four samples that were measured at less than 0.1 ng/ml by RIA (LLD = 0.1 ng/ml) to the Immulite® assay. Each of the samples read < 0.5 ng/ml which is the LLD for the Immulite® assay. In addition, four samples from the correlation study (Fig. 1),

Fig. 1. C-peptide results from hospitalized patients. Levels of circulating C-peptide in 30 hospitalized patients were determined by the DPC Immulite® C-peptide assay and plotted against those obtained with a comparable RIA method. The y -intercept is 0.88, slope 0.82, and r -value 0.97. The standard error of the estimate (Sy,x) = 0.56.

which read 0.2 ng/ml by RIA, read 0.7 ± 0.2 ng/ml by the Immulite® assay.

Based on the results obtained with serial dilutions of a high level (10.2 ng/ml) serum-based calibrator, the Immulite® C-peptide assay demonstrated good linearity, even at concentrations below 0.5 ng/ml, which is the recommended lower level of detection (LLD). Acceptable recoveries (87–112%) were observed throughout the range of concentrations tested. Our LLD for the Immulite® assay was determined to be 0.2 ng/ml with a confidence limit of 99% which is lower than the manufacturer's recommended LLD of 0.5 ng/ml.

Between-run precision was assessed at two different levels: 2.3 ng/ml and 10.2 ng/ml. The low control had a mean of 2.0 ng/ml with a coefficient of variation (CV) of 9% while the high control had a mean of 9.9 ng/ml and a CV of 8%. These results compare well with the RIA method, which has CVs for between-run precision ranging from 2 to 10% according to manufacturer.

Interference studies demonstrated less than a 10% change in C-peptide concentration for the concentrations of bilirubin, triglyceride, and hemoglobin tested (see Materials and Methods). The greatest single interference observed was +9.4% at a concentration of 9 g/l of hemoglobin.

DISCUSSION

Measurement of C-peptide may be used to determine if a patient's hypoglycemia is due to an endogenous cause such as insulinoma, or to an exogenous cause such as factitious hypoglycemia secondary to the administration of insulin. In the first scenario, C-peptide and insulin concentrations are elevated, whereas in the second, insulin concentrations are elevated and the C-peptide level is normal or below normal. In these types of cases, precision is not a major concern.

When following pancreatic β -cell function a more precise assay may be required. In order for a laboratory to implement an automated C-peptide method, it is essential for the assay to correlate with existing, accepted RIA methods. The Immulite® between day CV of 8–9% compares well with the 2–10% CV of the "gold standard" RIA. Also, the Immulite® sensitivity of 0.5 ng/ml is well below the normal C-peptide reference range (0.78–1.89 ng/ml, (9), which makes it suitable for assessing pancreatic β -cell function.

In terms of interference, DPC acknowledges a problem with elevated bilirubin, with approximate 20 and 35% increases in C-peptide values at 10 and 20 mg/dl bilirubin, respectively. DPC further states that hemolysis has no effect on this assay and lipemic samples should be cleared by centrifugation prior to analysis. Our results indicate that bilirubin, hemolysis, and lipemia up to a triglyceride concentration of 2,317 mg/dl have less than a 10% effect on C-peptide concentrations when spiked samples were compared to samples that had vehicle alone added. In the case of bilirubin, we observed that the vehicle alone had a significant ef-

fect on the result compared to a sample with no added vehicle, suggesting that the manufacturer's statement includes the effect of both vehicle and bilirubin.

The DPC Immulite® C-peptide assay offers significant advantages compared to RIA including improved analysis time and use of nonradioactive materials. In addition, because the method is fully automated it also reduces the amount of personnel time required to perform the test. We conclude that the DPC Immulite® chemiluminescent immunoassay for C-peptide is an acceptable alternative to RIA methodology. As a result, we have implemented this assay in our own laboratory.

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